Neuronal growth and interconnectivity

One of the main research directions in our group aims at understanding the basic physical principles that neuronal cells use to form functional connections with one another. The basic working unit of the brain is the neuron, a specialized cell designed to transmit information to other neurons, muscle, or gland cells. It consists of a cell body plus long threadlike axons that transmit electrical impulses, and shorter, thicker dendrites, which receive messages from other cells. A daunting task in biological physics is to figure out how as many as 100 billion neurons are produced, grow, and organize themselves into the truly wonderful information-processing machine which is the brain. To date the physical processes involved in forming functional neuronal connections (synapses), the mechanisms of axonal navigation to their target regions and their biomechanical interactions with proteins and guidance factors are still largely unknown. The main scientific goal of this research direction is to understand the fundamental processes governing the development of connections and communications between neurons in living systems by studying the growth and interconnectivity of small numbers of neurons patterned in simplified, well-controlled geometries. The central hypothesis explored is that simplifying the neuronal growth environment by creating highly controlled neuronal circuits in vitro will allow the basic rules that underlie neuronal development and the formation of neural connections to be elucidated.

We are using Atomic Force Microscopy (AFM) and nanotechnology to advance our physical understanding of biological processes. For example, one research direction aims at understanding the basic rules that neuronal cells use to form functional connections with one another. Our central hypothesis is that simplifying the neuronal growth environment by creating highly controlled neuronal circuits in vitro will allow the basic rules that underlie neuronal development and the formation of neural connections to be elucidated. We are developing an integrated approach that combines several experimental techniques (AFM nanolithography, AFM-based Electrical Force Microscopy, or EFM, and Fluorescence Spectroscopy) and theoretical methods (Fokker-Planck formalism, stochastic differential equations, continuum mechanics) with the aims of studying fundamental biophysical and biomechanical processes involved in neuronal growth and the formation of neuronal networks. On the experimental side this research is done in collaboration with Prof. David Kaplan (Tufts BME). These topics are of fundamental interest not only for biological physics but also for neuroscience and biochemistry, and as physicists we hope to bring both a novel set of experimental techniques and a distinctive intellectual approach to these highly complex and rapidly evolving fields.

![Fluorescence image of cortical neurons patterned on Au/laminin substrates.](image)

**Figure 1.** Left: Fluorescence image of cortical neurons patterned on Au/laminin substrates. The areas in red (labeled with fluorescent phalloidin) are marking the F-actin filaments. The blue regions show the cell body and neuronal processes: a long axon and several short dendrites. Right: Three-dimensional image superimposing the AFM topography and AFM-measured elastic modulus for the cell body of a live cortical neuron. The fluorescence image of the same cell (top right corner, the cell is stained with Tubulin Tracker Green) shows the regions inside the cell with high concentration of microtubules. The bright field image of the same cell is shown in the bottom-right corner (the image on the right is from the Biophys J paper).
During the past few years our research group has created neuronal networks on several different types of protein substrates, and we have used the Atomic Force Microscope (AFM) and Fluorescence Spectroscopy based techniques to characterize and explore the influence of biochemical and biomechanical cues on the growth and interaction of neuronal cells with surrounding guidance factors. Specifically, we have developed a unique approach that combines AFM topography, AFM force spectroscopy and Fluorescence Spectroscopy to systematically investigate the morphology, elastic properties, and real-time growth of neuronal processes in the presence of different types of extracellular matrix proteins and growth factors (Figure 1). We have created a series of systems containing well-controlled neuron geometries where the type of the underlying growth promoting protein is different from sample to sample. For each system we have measured key biomechanical parameters related to neuronal growth such as height and elastic modulus, and have determined how these parameters change both during neuron growth and upon disruption of internal components of the cell. Our recent results published in the *Biophysical Journal* represent the first systematic studies that compare biomechanical parameters between different neuronal cell types. Our paper also contains the highest resolution elasticity maps obtained on neuronal cell soma currently available in literature. In a different set of experiments published in *Physical Biology* we have measured elasticity maps of living cortical neurons as a function of temperature, and correlate these maps to the locations of internal structural components of the cytoskeleton. We found that neurons display a significant increase in the average elastic modulus upon a decrease in ambient temperature from physiological (37°C) to room (25°C) temperature. We have demonstrated that the dominant mechanism by which the elasticity of the neurons changes in response to temperature is the stiffening of the actin components of the cytoskeleton induced by the molecular motor myosin II.

In a different set of experiments, we have demonstrated that unidirectional nanotextured surfaces can bias neuronal growth through mechanical coupling between axons and the growth surfaces. This work was done in collaboration with Prof. Tim Atherton (Tufts) and Prof. Melik Demirel (Penn State), and the results have been published in *Applied Physics Letters*. In a very recent work published in *Physical Review E* we have reported on a general approach to describe axonal dynamics, based on the Fokker-Planck formalism. We have shown that axonal growth can be described as diffusion in an external potential, representing the collective contributions of all causal influences on the growth cone. Furthermore, we have used this approach to obtain effective growth rules that reveal emergent regulatory mechanisms for axonal pathfinding on 2-dimensional substrates. We are currently working on extending these studies for other types of surfaces, in order to investigate the interplay between biomechanical, geometrical and biochemical cues, and the role that these factors play in the formation of neuronal networks.

Figure 2. (a) *Top*: Schematic of the axon (growth cone) adhesion to the asymmetric (nano-ppx) surface. Fluorescence (*middle*) and bright filed (*bottom*) images of two different cells growing on the surface. The cells extend axons preferentially in the direction opposite to the direction of the tilted nanorods. (b) Scanning Electron Microscope (*top*) and AFM (*bottom*) images of a nano-ppx surface (*image from the APL paper*).
Figure 3. Angular distribution of axonal growth. (a) Definition of the angles (0 radians represents the direction opposite to the direction of the nanorods, see Fig. 2). (b) Measured angular distribution on a control surface (PDL/glass). (c)-(e) Measured angular distributions of axonal growth on nano-pxx surfaces with cells cultured at different densities: (c) 2000, (d) 6000 and (e) 25000, cells per square centimeter, respectively. Solid lines in the right-hand column represent fits to the data using a general model based in the Fokker-Planck formalism (image from the APL paper).

We have recently started a collaboration with researchers from the Oak Ridge National Laboratory (ORNL), which aims at developing novel techniques for measuring electrical signaling between neuronal cells and the activity of ion channels. Specifically, we will use the voltage biased AFM - tip as a very sensitive/minimally invasive local electrode (gate) to control the activity of various ion channels along the length of the axon/dendrite. The focus of these studies is to gain a deeper quantitative description of the basic physical processes involved in the dynamics of ion channels when electrical signals (action potentials) are transmitted between different neurons. We also seek to measure under what conditions synaptic junctions are functional and to learn what cues influence neurons to form functional synapses. In addition to their importance for understanding fundamental biophysical processes, these studies may lead to novel insights into diseases that result when these neuronal processes fail, including birth defects, mental disorders, and sensory-motor deficits.